Description

Dendrite Elongation Inhibitor for Melanocyte and Skin Preparation for External Use containing the same

Technical Field

The present invention relates to a dendrite elongation inhibitor for melanocytes and a skin preparation for external use containing the dendrite elongation inhibitor for melanocytes as an active ingredient.

Background Art

Keeping skins fair and beautiful is what many women hope, and many whitening cosmetics have therefore been developed. For example, whitening cosmetics can be exemplified by cosmetics containing ascorbic acid or a derivative thereof, kojic acid or a derivative thereof, tranexamic acid or a derivative thereof, hydroquinone glycoside, or the like. However, most of the cosmetics have a mechanism utilizing the action of inhibiting tyrosinase and inhibiting the biosynthesis of melanin, and we had to say that there is a limit on its effect. That is, even though the whitening cosmetics containing those ingredients as active ingredients are effective for symptoms such as age spots, freckles, and dark complexion that result from the abnormally accelerated production of melanin, we had to say that such whitening cosmetics do not have much effect

on dyschromatosis to which the amount of melanin produced less contributes. In other words, there exists dyschromatosis for which tyrosinase inhibitors are not or less effective, and it has been desired that means for alleviating such dyschromatosis is developed.

On the other hand, examples of dyschromatosis to which the amount of melanin produced less contributes include those resulting from the accelerated migration of melanin granules from melanocytic dendrites. Although it is considered for such dyschromatosis to treat by inhibiting the elongation of dendrites that occurs when melanocytes allows melanin granules to migrate, not so many whitening agents utilizing such a mechanism have been known. That is, it can be said that there has been a demand for the development of whitening agents utilizing such a mechanism.

Achillea millefolium L. that is a source plant from which the inventors of the present invention have found Centaureidin (5,7-dihydroxy-3,6-dimethoxy-2-(5-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-on; hereinafter also referred to as "Compound 1") that is a compound represented by the general formula (1). It has already been known to have its extract useful as a humectant for cosmetics (JP-A 02-172907), to be useful in the stabilization of kojic acid in cosmetics (JP-A 07-17848), to have action of inhibiting tyrosinase (JP-A 08-104646), to have action of eradicating active oxygen (JP-A 11-246336), to have action of inhibiting α-MSH (JP-A 11-349435), and so on. However, it has not been known at all that

Centaureidin inhibits the elongation of melanocytic dendrites and that it is useful for alleviating, by such action, dyschromatosis on which melanin production inhibitors utilizing usual tyrosinase inhibitory action are not or less effective.

Moreover, the compound represented by the general formula (1) such as Centaureidin has already been known:

1) to be incorporated in plants of the genus Artemisia and useful for treating allergic diseases (published international application WO 20020419109);

2) to has anti-cancer action (US patent No. 493540); and

3) to be incorporated in plants of the genus Centaurea cyanus (Flamini Guido et. al., Phytochemistry, 58(8), 1229-1233, 2001).

However, it has not been known at all that such a substance is incorporated in Achillea millefolium L. of the family Asteraceaeis. It has not been known in the least that the substance inhibits the elongation of melanocytic dendrites, and that it is useful for alleviating, by such action, dyschromatosis on which melanin production inhibitors utilizing usual tyrosinase inhibitory action are not or less effective.

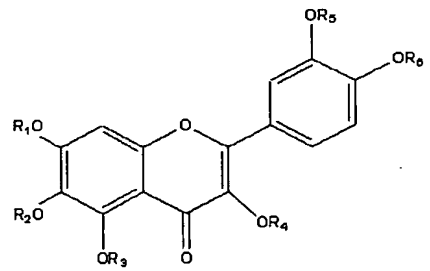
Disclosure of the Invention

The present invention has been achieved under such circumstances, and an object of the present invention is to provide a useful ingredient for inhibiting the elongation of melanocytic

dendrites and alleviating, by this action, dyschromatosis on which melanin production inhibitors utilizing usual tyrosinase inhibitory action are not or less effective.

In light of such circumstances, the inventors of the present invention have conducted extensive studies and redoubled efforts to acquire a useful ingredient for inhibiting the elongation of melanocytic dendrites and alleviating, by this action, dyschromatosis on which melanin production inhibitors utilizing usual tyrosinase inhibitory action are not or less effective. As a result, the inventors of the present invention have completed the present invention by finding out that a compound represented by the general formula (1) and/or a salt thereof, which is incorporated in Achillea millefolium L. of the family Asteraceaeis have (has) such action. Namely, the present invention relates to a technique shown below.

(1) A dendrite elongation inhibitor for melanocytes consisting of a compound represented by the following general formula (1):



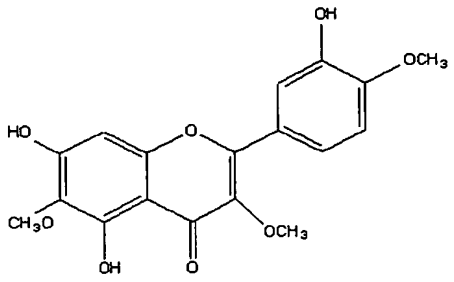
formula (1)

and/or a salt thereof,

wherein R_1, R_2, R_3, R_4, R_5, and R_6 each independently represent

a hydrogen atom or a C_{1-4} alkyl group.

(2) The dendrite elongation inhibitor for melanocytes according to (1), characterized in that the compound represented by the general formula (1) is Centaureidin indicated by the following formula.



(3) A skin preparation for external use for inhibiting elongation of melanocytic dendrites, comprising the dendrite elongation inhibitor for melanocytes according to (1) or (2) as an active ingredient.

(4) The skin preparation for external use for inhibiting elongation of melanocytic dendrites according to (3), characterized in that the skin preparation for external use is used for alleviating dyschromatosis on which tyrosinase inhibitors have insufficient effect.

(5) The skin preparation for external use for inhibiting elongation of melanocytic dendrites according to (3) or (4), characterized in that the skin preparation for external use is a cosmetic.

Best Mode for carrying out the Invention

(1) Dendrite elongation inhibitor for melanocyte of the present invention

A dendrite elongation inhibitor for melanocytes of the present invention consists of a compound represented by the above-described general formula (1) and/or a salt thereof.

In the general formula (1), R_1, R_2, R_3, R_4, R_5, and R_6 each independently represent a hydrogen atom or an alkyl group.

The alkyl group is preferably a C_{1-4} alkyl group, and examples thereof include a methyl group, an ethyl group, a propyl group, a 1-methylethyl group, a n-butyl group, a 1-methylpropyl group, a 2-methylpropyl group, and a 1,1-dimethylethyl group. Of those, particularly preferred is a methyl group.

The compound represented by the general formula (1) can preferably be exemplified by Centaureidin.

Such a compound represented by the general formula (1) can be directly used, or can be used in a salt form after treatment with alkali.

The salt can be applied without particular limitation as long as it is physiologically acceptable, and can preferably be exemplified by alkali metal salts such as sodium salts and potassium salts, alkaline-earth metal salts such as calcium salts and magnesium salts, ammonium salts, organic amine salts such as triethanolamine salts, and triethylamine salts, and basic amino acid salts such

as lysine salts and arginine salts. Particularly preferred are alkali metal salts, which are easy to be prepared.

In a skin preparation for external use of the present invention, the compound represented by the general formula (1) and/or the salt thereof can be incorporated alone or a combination of two or more kinds of them can be incorporated.

Such a compound represented by the general formula (1) and/or a salt thereof may be purified one, and may be an extract from a plant or a fraction thereof, or the like containing an effective amount of the compound represented by the general formula (1) and/or the salt thereof.

Plants of the genus Achillea sp. of the family Asteraceaeis, preferably Achillea millefolium L. of the family Asteraceaeis can be used as such plants. A plant used in the extraction of the compound represented by the general formula (1) and/or the salt thereof may be the entire plant, a part of the plant containing the compound represented by the general formula (1) and/or the salt thereof, or a processed product of the plant. For example, an extract of the above-ground part of the genus Achillea millefolium L. of the family Asteraceaeis can be purified and fractionated to obtain the compound represented by the general formula (1) and/or the salt thereof. The compound represented by the general formula (1) and/or the salt thereof can be identified by X-ray analysis or the like.

The extract can particularly preferably be exemplified by an

extract with a highly polar solvent. The highly polar solvent can preferably be exemplified by: ethers such as diethyl ether, isopropyl ether, and tetrahydrofuran; halogenated hydrocarbons such as methylene chloride and chloroform; esters such as ethyl acetate and methyl formate; ketones such as acetone and methylethylketone; nitriles such as acetonitrile; alcohols such as 1,3-butanediol, ethanol, and isopropyl alcohol; and water. Of those, alcohols are particularly preferred. It is noted that the above-described solvent may be one kind or a mixture of two or more kinds of them.

Extraction may typically be carried out by adding 1 to 10 times by weight of a solvent with respect to the entire plant or a part of the plant, followed by a few-day immersion if carried out at room temperature or a few-hour immersion if carried out around a boiling point. After extraction, the solvent can be removed by vacuum concentration or the like, if necessary. The compound represented by the general formula (1) can be isolated from the extract from which solvent has been removed, by liquid-liquid extraction with ethyl acetate and water, and the like, or purification by silica gel column chromatography using, for example, chloroform-methanol as an eluting solvent, or the like.

A preferable content of the compound represented by the general formula (1) and/or the salt thereof in a skin preparation for external use of the present invention is 0.001 to 10% by weight, more preferably 0.005 to 5% by weight with respect to the total amount of the skin

preparation for external use. This is because, if the content is too small, inhibitory action on the elongation of melanocytic dendrites may not be exhibited; while, if the content is too large, the action may level off and may unnecessarily inhibit the degree of freedom of a prescription.

(Example of production)

Ten kilograms of a dried product of the above-ground part of the genus Achillea millefolium L. of the family Asteraceaeis) was cut into narrow pieces, which were then added to ethanol 501 and heated to reflux for 3 hours. After cooled to room temperature, the resulting mixture was concentrated under vacuum concentration, and 11 of ethyl acetate and water were added thereto. The resulting mixture was subjected to liquid-liquid extraction to take out the phase of ethyl acetate, followed by vacuum concentration to prepare an extract. After dissolved in chloroform, the residue was charged on silica gel column chromatography and purified with an eluting solvent chloroform:methanol=100:1 to 70:30 to give 211.5 mg of Compound 1. The structure was determined by X-ray analysis.

(2) Skin preparation for external use of the present invention

A skin preparation for external use of the present invention is characterized by containing the above-described dendrite elongation inhibitor for melanocytes of the present invention. A skin preparation for external use used herein means a general term

for compositions applied for external use for skins, and can be exemplified by cosmetics including quasi-drugs, dermatologic drugs for external use, and dermatologic sundry articles for external use. Of those, particularly preferred are cosmetics. This is because the above-described dendrite elongation inhibitor for melanocytes of the present invention has excellent safety, so that the dendrite elongation inhibitor for melanocytes can be used continually and habitually as cosmetics, and more satisfactorily exhibit whitening action in such a usage pattern.

The dosage forms of cosmetics are not particularly limited and the cosmetics can be used not only in emulsified dosage forms such as cream and milky lotions but in solution dosage forms such as skin lotions and essences, because the dendrite elongation inhibitor of the present invention has particularly high physical properties of polarity.

Skin preparation for external use of the present invention can contain the optional ingredients used generally in a skin preparation for external use, beside the dendrite elongation inhibitor for melanocytes described above. Preferable examples of the optional ingredients include: hydrocarbons such as squalene, liquid paraffin, light-gravity liquid isoparaffin, heavy-gravity liquid isoparaffin, microcrystalline wax, and solid paraffin; silicones such as dimethycon, femethycon, cyclomethycon, amodimethycon, polyether denatured silicone; esters such as jojoba

oil, carnauba wax, haze wax, bees wax, spermaceti wax, octyldodecyl oleate, isopropyl myristate, neopentyl glycol diisostearate, and malic diisostearate; aliphatic acids such as stearic acid, lauric acid, myristic acid, palmitic acid, isostearic acid, isopalmitic acid, behenic acid, and oleic acid; higher alcohols such as behenyl alcohol(1-docosanol), cetanol, oleyl alcohol, and octadecyl alcohol; triglycerides such as castor oil, coconut oil, hydrofined coconut oil, camellia oil, wheat germ oil, isostearate triglyceride, isooctanoate triglyceride, and olive oil; polyhydric alcohols such as 1,3-butanediol, glycerin, diglycerin, dipropylene glycol, polyethylene glycol, 1,2-pentandiol, 1,2-hexylene glycol, and isoprene glycol; nonionic detergents such as sorbitan sesquiolate, sorbitan monooleate, sorbitan trioleate, sorbitan sesquistearate, sorbitan monostearate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene stearate, polyoxyethyleneoleate, polyoxyethylene glyceril fatty ester, polyexyethylene alkyl ether, and polyoxyethylene hardened castor oil; anionic detergents such as sodium lauryl stearate, polyoxyethylene alkyl sulfate, and sulfosuccinate; cationic detergents such as quaternary alkyl ammonium salt; ampholytic detergents such as alkyl betaine; organic powders such as crystalline cellulose, crosslinking type methylpolysiloxane, polyethylene powder, and acrylic resin powder; powders that can be surface-treated such as talc, mica, sericite, magnesium carbonate, calcium carbonate,

titanium dioxide, iron oxide, iron blue, ultramarine, titanic mica, titanic sericite, and silica; thickening agents such as alkyl acrylate-alkyl methacrylate copolymer and/or a salt thereof, carboxyvinyl polymer and/or a salt thereof, xanthan gum, and hydroxypropyl cellulose; active ingredients such as vitamins, terpenes, and steroids; examples of vitamins include retinol, retinoic acid, tocopherol, riboflavin, pyridoxin, ascorbic acid, and ascorbic phosphate; examples of terpenes include glycyrrhizic acid salt, glycyrrhetin, ursolic acid, and oleanolic acid; examples of steroids include estradiol, ethynilestradiol, and estriol; antiseptic agents such as phenoxyethanol, parabens, Hibitane Gluconate, and benzalkonium chloride; and UV absorbing agents such as dimethylamino benzoate, cinnamates, and benzophenones.

Of course, a whitening agent having a different mechanism from that of the dendrite elongation inhibitor of the present invention, for example, ascorbic acid or a derivative thereof, kojic acid or a derivative thereof, tranexamic acid or a derivative thereof, hydroquinone glycoside, or the like, can also be incorporated in the skin preparation for external use. Incorporating such a whitening agent gives at least a synergistic effect and is therefore preferred. A preferable content of such a whitening agent having a different mechanism from that of the dendrite elongation inhibitor of the present invention is 0.01 to 5% by weight in total with respect to the total amount of the skin preparation for external use.

Applicable disease of the skin preparation for external use of the present invention can also preferably be exemplified by dyschromatosis on which tyrosinase inhibitors have insufficient effects. "Dyschromatosis on which tyrosinase inhibitors have insufficient effects" used herein means dyschromatosis judged by 70% or more panelists to be "dyschromatosis having no alleviation" when tested by a method described in Example 2 or the like using a tyrosinase inhibitor (e.g., arbutin).

The skin preparation for external use of the present invention can be produced by treating the above-described essential ingredient and an optional ingredient according to a standard method.

Examples

Although the present invention will more fully be described hereinafter with reference to Examples, it is understood that the present invention is not intended to be limited only to such Examples.

<Example 1>

According to a method shown below, inhibitory action on the elongation of dendrites was examined using human melanocytes.

(Reagent, etc.) Cells, basal media, and amplification additives were purchased from KURABO INDUSTRIES LTD.

(Cell) Normal human melanocyte

(Medium) Basal medium (Medium 154S) supplemented with reagents described below

(Reagent) Amplification additive: bovine pituitary extract (BPE) (final concentration of 0.4% v/v in the medium), fetal bovine serum (FBS) (final concentration of 0.5% v/v in the medium), human recombinant basic fibroblast growth factor (rFGF-B)(final concentration of 3 ng/ml in the medium), hydrocortisone (final concentration of 0.18 μg/ml in the medium), insulin (final concentration 5 μg/ml in the medium), transferrin (final concentration of 5 μg/ml in the medium), phorbol 12-myristate 13-acetate (PMA) (final concentration of 10 ng/ml in the medium), heparin (final concentration of 3 μg/ml in the medium), and PSA solution (mixture solution of penicillin concentration of 50,000 Unit/ml, streptomycin concentration of 50 μg/ml, and amphotericin B concentration of 12.5 μg/ml; 1-ml addition with respect to 500 ml of the medium)

(Method)

The extract of Achillea millefolium L. and Compound 1 (Centaureidin) obtained in the above-described example of production were diluted in a basal medium so that the concentration of Centaureidin was brought up to 100 μg/ml, to make a sample solution. It is noted that a control is a solution having only a basal medium.

Normal human melanocytes were inoculated into a 48-well microplate (3,000 cells/well, 200 μl medium) and cultured at 37°C.

After 24 hours, 50 μl of the sample solution was added thereto.

After 24 hours of the addition of the sample solution,

inhibition against the elongation of dendrites was observed.

(Result)

The result is shown in Table 1 by the length of the dendrite. It is seen that the dendrite is elongated in the control by the effect of adding the growth factor, while elongation is inhibited in the added group of Centaureidin.

Table 1

Added compound	Length of dendrite(μm)
Centaureidin	26± 8
Extract of Achillea millefolium L.	108±21
Control	140±29

<Example 2>

According to a prescription shown below, a cosmetic that was a skin preparation for external use of the present invention was prepared. That is, ingredients of I, II, and III each were heated to 70°C. II was neutralized with III and emulsified by gradually adding I with stirring. The resulting mixture was homogenized with a homogenizer, followed by cooling with stirring to give a milky lotion. Comparative Example 1 in which Compound 1 in this prescription was substituted by squalene was made. Twenty persons in total (10 persons for 1 group) suffering from dark complexion that was not alleviated by usual cosmetics for inhibiting the production of melanin were used to examine the degree of alleviation

of dark complexion in a usage test with use at twice in the morning and evening for 30 consecutive days. The degree of alleviation was evaluated after 30-day use by scores of Score 5: significantly alleviated, Score 4: obviously alleviated, Score 3: alleviated, Score 2: slightly alleviated, and Score 1: not alleviated. The result is shown in Table 2. This reveals that the cosmetic that is the skin preparation for external use of the present invention has excellent whitening effect.

I	
Squalene	10 parts by weight
Sorbitan sesquistearate	2 parts by weight
Compound 1	0.05 part by weight
Butylparaben	0.1 part by weight
II	
1,3-buthanediol	5 parts by weight
Xanthan gum	0.1 part by weight
Acrylate alkyl-methacrylate alkyl (C10-30)	0.4 part by weight
Methylparaben	0.1 part by weight
Water	50 parts by weight
III	
Potassium hydroxide	0.2 part by weight
Water	32.05 parts by weight

Table 2

Sample	Score 5	Score 4	Score 3	Score 2	Score 1
Example 2		4	4	2	
Comparative Example 1				2	8

<Example 3>

A skin preparation for external use (cosmetic) was made in the same way as in Example 2 except that the amount of Compound 1 was changed, and similarly evaluated using 10 similar panelists. Similar effect was observed in this skin preparation for external use.

I	
Squalene	10 parts by weight
Sorbitan sesquistearate	2 parts by weight
Compound 1	0.1 part by weight
Butylparaben	0.1 part by weight
II	
1,3-buthanediol	5 parts by weight
Xanthan gum	0.1 part by weight
Arylate alkyl-methacrylate alkyl (C10-30)	0.4 part by weight
Methylparaben	0.1 part by weight
Water	50 parts by weight

III

Potassium hydroxide	0.2 part by weight
Water	32.0 parts by weight

Table 3

Sample	Score 5	Score 4	Score 3	Score 2	Score 1
Example 3		5	4	1	

<Example 4>

According to a prescription shown below, a skin preparation for external use (cosmetic) was made in the same way as in Examples 2 and 3, and similarly evaluated using similar panelists. Comparative Example 2 in which Compound 1 was substituted by arbutin was made and similarly evaluated. The results are shown in Table 4. This reveals that less whitening effect of the tyrosinase inhibitor was observed in the panelists and that the dendrite elongation inhibitor for melanocytes of the present invention was observed to effectively act even in such panelists.

I

Squalene	10 parts by weight
Sorbitan sesquistearate	2 parts by weight
Compound 1	1 part by weight
Butylparaben	0.1 part by weight

II

1,3-buthanediol	5 parts by weight
Xanthan gum	0.1 part by weight
Acrylate alkyl-methacrylate alkyl (C10-30)	0.4 part by Weight
Methylparaben	0.1 part by weight
Water	50 parts by weight
III	
Potassium hydroxide	0.2 part by weight
Water	31.1 parts by weight

Table 4

Sample	Score 5	Score 4	Score 3	Score 2	Score 1
Example 3	1	6	3		
Comparative Example 2				3	7

Industrial Applicability

According to the present invention, a useful ingredient for inhibiting the elongation of melanocytic dendrites and alleviating, by this action, dyschromatosis on which melanin production inhibitors utilizing usual tyrosinase inhibitory action are not or less effective can be provided.